

1        1. A method for identifying a drug candidate for promoting tissue-specific  
2        differentiation of a stem cell, the method comprising the steps of:

3            (A) providing a library of test substances, the library comprising at least a first test  
4        substance and a second test substance, the first and second test substances having different  
5        molecular structures;

6            (B) providing an in vitro culture of stem cells, the culture being divided into at  
7        least a first subculture and a second subculture;

8            (C) contacting the first subculture with the first test substance and the second  
9        subculture with the second test substance;

10           (D) culturing the first and second subcultures respectively contacted with the first  
11        and second test substances under conditions that would promote tissue-specific differentiation  
12        of the stem cells if an agent that promoted tissue-specific differentiation was in contact with  
13        the stem cells; and

14           (E) analyzing the cells in the first and second subcultures for increased tissue-  
15        specific gene expression.

1        2. The method of claim 1, wherein the stem cells are embryonic stem cells.

1        3. The method of claim 2, wherein the embryonic stem cells are mammalian  
2        embryonic stems cells.

1        4. The method of claim 3, wherein the mammalian embryonic stem cells are  
2        murine embryonic stems cells.

1        5. The method of claim 4, wherein the murine embryonic stem cells R1  
2        embryonic stems cells.

1           6. The method of claim 3, wherein the mammalian embryonic stem cells are  
2 human embryonic stems cells.

1           7. The method of claim 1, wherein the conditions that would promote tissue-  
2 specific differentiation of the stem cells comprises culturing the first and second subcultures  
3 in a differentiating medium.

1           8. The method of claim 1, wherein the conditions that would promote tissue-  
2 specific differentiation of the stem cells comprises culturing the first and second subcultures  
3 at about 37°C.

1           9. The method of claim 1, wherein the conditions that would promote tissue-  
2 specific differentiation of the stem cells comprises culturing the first and second subcultures  
3 in a humidified, carbon-dioxide containing incubator.

1           10. The method of claim 1, wherein the conditions that would promote tissue-  
2 specific differentiation of the stem cells comprises culturing the first and second subcultures  
3 for a time period of at least five days.

1           11. The method of claim 10, wherein the time period is at least seven days.

1           12. The method of claim 11, wherein the time period is between seven and  
2 eighteen days.

1           13. The method of claim 1, wherein the first and second subcultures are cultured  
2 in a microtiter plate.

1           14. The method of claim 1, wherein the step (E) of analyzing the cells in the first  
2 and second subcultures for increased tissue-specific gene expression comprises isolating  
3 mRNA from the first and second subcultures.

1           15. The method of claim 14, wherein total cellular RNA is isolated from the first  
2 and second subcultures.

1           16. The method of claim 14, wherein the step (E) further comprises reverse-  
2 transcribing the mRNA to create cDNA.

1           17. The method of claim 1, wherein the step (E) of analyzing the cells in the first  
2 and second subcultures for increased tissue-specific gene expression comprises performing a  
3 polymerase chain reaction (PCR).

1           18. The method of claim 14, wherein the isolated mRNA is immobilized on a  
2 substrate.

1           19. The method of claim 18, wherein the substrate is contacted with a probe that  
2 specifically hybridizes to the tissue-specific mRNA.

1           20. The method of claim 1, wherein the step (E) of analyzing the cells in the first  
2 and second subcultures for increased tissue-specific gene expression is performing using gene  
3 chip technology.